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CHARACTERISTICS OF MONOAMINE OXIDASES IN BRAIN AND OTHER ORGANS OF THE GOLDEN HAMSTER

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Abstract—The activities of monoamine oxidase (MAO) were measured with the type A substrate, serotonin, and the type B substrate, phenylethylamine (PE), in brain, heart, kidney, lung, liver and spleen of the golden hamster, rat and rabbit. The relative activities measured with these two substrates varied markedly between different organs of the same species and between the same organ of different species. The rabbit had the lowest A/B ratio for each of the organs examined. The A/B ratios in the hamster were comparable to those of the rat for each of the organs, except for a higher ratio in the brain and a lower ratio in the heart. The activity measured with PE for hamster brain was only 7 and 8 per cent of the corresponding activity in the brain of the rat and rabbit, respectively. Studies using the selective inhibitors, clorgyline and deprenyl, in combination with selective substrates, indicated that the small amount of activity in hamster brain towards the substrate PE was, indeed, due to MAO-B rather than a nonspecific action of MAO-A. The advantages of this method for the detection of multiple forms of MAO, particularly when the amount of one form is very small as compared with the other, are discussed.

Johnston [1] originally distinguished two types of monoamine oxidase (MAO: E.C. 1.4.3.4) on the basis of biphasic inhibition curves produced by the irreversible inhibitor, clorgyline [*N*-methyl-*N*-propargyl-3-(2,4-dichlorophenoxy)-propylamine]. According to his nomenclature, type A MAO was that activity inhibited by low concentrations of the drug whereas type B MAO was inhibited only when the clorgyline concentrations were increased several orders of magnitude [2].

Evidence that these types A and B MAO represent physiologically distinct enzymatic forms is shown by the fact that striking differences in their relative activities are found between animal species or between organs of the same species [3, 4]. The proportion of type A activity determined from the plateau of clorgyline inhibition curves varies in the rat from 15 per cent in the pineal gland to 95 per cent in the spleen [3]. Squires [4] compared the relative amounts of A and B forms of MAO in various organs of eight species and found that the A/B ratio varies considerably in different species. In contrast to the rat, the livers of mouse and rabbit appear to be essentially devoid of MAO-A [4]. Edwards and Chang [5] reported that rabbit platelets contain both A and B MAO, in contrast to human platelets, which contain only the B enzyme.

In the present study, we have examined the characteristics of MAO in various organs of the hamster and compared them to MAOs in the rat and rabbit. In contrast to any species previously examined, the hamster brain exhibits very little MAO-B activity.

MATERIALS AND METHODS

Animals. Adult male golden hamsters (Mesocricetus auratus) were obtained from Lakeview Hamster Colony, Newfield, NJ (outbred strain, LVG:LAK). These animals were housed individually under a 14/10 light/dark cycle with lights off at 10 a.m. and provided with a diet of rat chow and fresh cabbage. Male albino rabbits and Sprague–Dawley rats were obtained from Hilltop Labs (Scottsdale, PA) and Zivic-Miller (Pittsburgh, PA), respectively.

Organ distribution of MAO. Rats and hamsters were killed by decapitation and the organs were rapidly removed and placed on ice. Rabbits were anesthetized with ether and bled to death. All organs were weighed and homogenized in 19 volumes of glass-distilled water using a Polytron (Brinkmann Instruments). Blood platelets were obtained from hamsters according to the procedures described by Odell and McDonald [6].

MAO assays. MAO was assayed with the substrates serotonin (5-HT) and PE, using the radiochemical procedures described by Edwards and Chang [5]. All assays were performed in duplicate for each substrate. Under the conditions used, the assays were linear during incubation at 37° for at least 1 hr, providing no more than 10 per cent of the substrate was converted to product. Unless otherwise indicated, the concentrations of 5-HT and PE were 100 and 4.88 μM, respectively.

Drug inhibition studies. Selective MAO inhibitors were used to further characterize hamster brain MAO activity. Clorgyline.HCl and deprenyl.HCl were dissolved in distilled water and added to the assay tubes already containing enzyme and buffer. Eleven different concentrations for each inhibitor were used to give final concentrations over the range 10^{-9}–10^{-4} M. The reactions were initiated by the addition of substrate 5 min after the inhibitor was added. Final volumes of the assay mixtures were 2 ml.

Enzyme kinetic studies. For the determination of the kinetic parameters, hamster whole brain homo-

Table 1. MAO-A and MAO-B activity in various organs of hamster, rat and rabbit*

Organ	MAO-A			MAO-B			Ratio MAO-A:MAO-B		
	Hamster	Rat	Rabbit	Hamster	Rat	Rabbit	Hamster	Rat	Rabbit
Brain	6.25	11.6	2.27	0.20	2.69	2.47	31.2	4.3	0.92
Heart	2.92	11.1	0.58	0.29	0.25	0.67	10.2	45.5	0.87
Kidney	10.7	7.02	3.64	0.95	1.01	3.28	11.3	7.0	1.1
Lung	8.82	4.90	1.53	1.64	2.31	1.37	5.4	2.1	1.1
Liver	8.68	32.4	1.80	6.26	17.3	4.83	1.4	1.9	0.37
Spleen	3.48	2.78	1.29	0.40	0.25	0.86	8.7	11.0	1.5

* Enzymatic activities are expressed as μmoles product formed/hr/g tissue. MAO-A and MAO-B were measured with the substrates 5-HT and PE, respectively.

genates were used and MAO activity was measured over the range of substrate concentration, 0.304–19.5 μM for PE and 54–434 μM for benzylamine. The values for K_m and V_{max} were determined graphically from Lineweaver–Burk plots. Since MAO exhibited marked substrate inhibition when PE concentrations were greater than 5 μM, only substrate concentrations below this value were used in the determination of K_m.

Materials. Phenylethylamine.HCl and benzylamine.HCl were obtained from K and K Laboratories (Plainview, NY). Serotonin creatinine sulfate was purchased from Sigma Chemical Co. (St. Louis, MO). Phenylethylamine.HCl, β-ethyl-[1-^{14}C] (9.86 mCi/m-mole) and serotonin binoxalate [2-^{14}C] (53 mCi/m-mole) were obtained from New England Nuclear (Boston, MA). Benzylamine-[1-^{14}C] (2.98 mCi/m-mole) was obtained from California Bionuclear Corp. (Sun Valley, CA). All reagents were obtained from Fisher Scientific Co. (Pittsburgh, PA).

Clorgyline was a gift from May and Baker, Ltd. (Dagenham, England). Deprenyl was kindly provided by Dr. J. Knoll (Budapest).

RESULTS

MAO-A and MAO-B activities in various organs of the hamster, rat and rabbit. The enzymatic activities of types A and B MAO, as determined with the substrates 5-HT and PE, respectively, and expressed as μmoles/hr/g wet wt of tissue, are given in Table 1 for six different organs of the hamster, rat and rabbit.

The most striking feature of these data is the extremely low activity of the B-form of MAO in hamster brain. This activity was only 7.4 per cent of the activity in rat brain and only 8.1 per cent of that in rabbit brain. In contrast, the activity of type A MAO in the hamster brain was 53.9 and 275 per cent of the corresponding activities in the rat and rabbit brain, respectively. The low activity of the B type of MAO was limited to the brain, since the activity of this enzyme in all other organs was comparable to that found in both other species.

As a result of the low activity of type B MAO in the hamster brain, the MAO A/B ratio is much higher in the hamster brain (31.2) than in either rat (4.3) or rabbit (0.92) brain. The A/B ratios in the hamster and rat differ by approximately 2-fold or less in all other organs examined, except in the heart, in which this ratio is approximately 4.5-fold higher in the rat than in the hamster. The data obtained for the rabbit organs demonstrate that this species has a much lower activity of the type A enzyme. The A/B ratios in the rabbit are close to unity (0.87–1.1) in the brain, heart, kidney and lung and somewhat higher in the spleen (1.5). This ratio is lowest in rabbit liver (0.37), where the type B enzymatic activity exceeds the type A activity.

The effects of clorgyline and deprenyl on hamster brain MAO as measured with 5-HT and PE as substrates. In view of the very low rate at which hamster brain homogenates are able to deaminate PE as compared to 5-HT, it was important to determine whether this activity was due to non-specific deamination by MAO-A or to the presence of a small amount of MAO-B activity. Consequently, we examined the characteristics of this activity in the presence of selective inhibitors of types A and B MAO.

As can be seen from Fig. 1, clorgyline was a relatively weak inhibitor of PE deamination ($I_{50} = 4.8 \times 10^{-7}$ M) as compared to 5-HT deamination ($I_{50} = 2.2 \times 10^{-9}$ M). These I_{50}'s differ by 220-fold. The differential effects of clorgyline on PE and 5-HT deamination clearly show the existence of two forms of MAO in the hamster brain. Furthermore, these data are consistent with the substrate specificities and clorgyline sensitivity of types A and B MAO in other species [2].

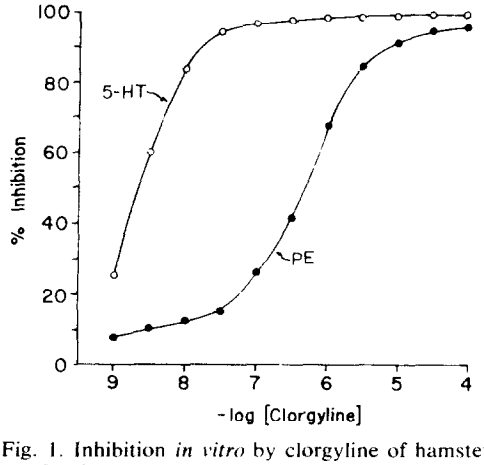

Fig. 1. Inhibition *in vitro* by clorgyline of hamster brain MAO using 5-HT (○) and PE (●) as substrates. Each assay tube contained 50 μl or 100 μl of whole brain homogenate (1:20) when 5-HT or PE, respectively, were used as substrates. Varying amounts of clorgyline were added to make the final concentration 10^{-9}–10^{-4} M, and the reaction was initiated by the addition of substrate.

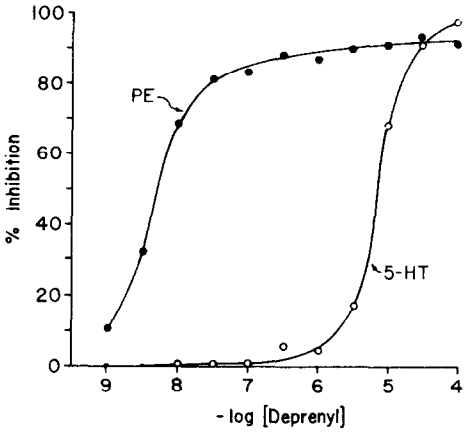

Fig. 2. Inhibition *in vitro* by deprenyl of hamster brain MAO using 5-HT (○) and PE (●) as substrates. Each assay contained 100 μl of whole brain homogenate (1:20) and reactions were carried out as in Fig. 1.

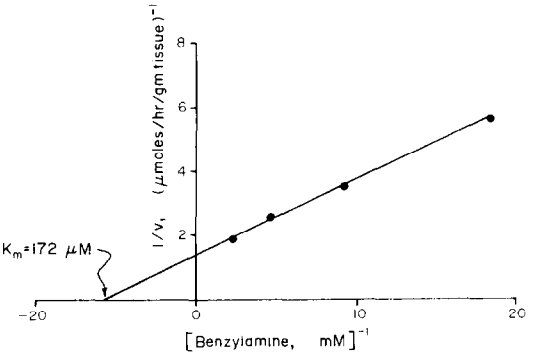

Fig. 4. Lineweaver–Burk plot for hamster whole brain homogenates using the substrate, benzylamine, over the range of 54.3–434 μM final concentrations.

Similar studies carried out with deprenyl again were consistent with the notion that PE deamination by the hamster brain was due to the activity of type B MAO. Very low concentrations of deprenyl ($I_{50} = 5.0 \times 10^{-9}$ M) produced inhibition of PE deamination but were without effect on 5-HT deamination (Fig. 2). Inhibition of 5-HT deamination occurred at only relatively high concentrations of deprenyl ($I_{50} = 6.8 \times 10^{-6}$ M) or 1360-fold higher than the corresponding I_{50} observed when PE was used as the substrate.

As judged from these I_{50} values, clorgyline was 1/96 as potent as deprenyl in blocking PE deamination but 3100-fold more potent in blocking 5-HT deamination. Approximately 5 per cent of the MAO activity measured with PE was not inhibited by even high concentrations of either deprenyl or clorgyline (Figs 1 and 2). This activity represents a very small amount of absolute enzymatic activity, since the total amount of activity with PE as substrate was only about 3 per cent of that with 5-HT as substrate (Table 1). Essentially all of the enzymatic activity measured with the substrate 5-HT was inhibited by 10^{-4} M concentration of clorgyline or deprenyl.

Kinetic constants for hamster brain MAO using the B-specific substrates, PE and benzylamine. In order to further characterize the enzyme responsible for PE deamination by hamster brain independent of the drug inhibition studies, the Michaelis–Menten kinetic constants for PE and benzylamine, another type B-specific substrate, were determined. As shown in Figs 3 and 4, the Lineweaver–Burk plots of these data were linear, except at the highest concentrations of PE (> 5 μM) where a deviation from linearity was apparently due to substrate inhibition. The apparent K_m's for PE and benzylamine were 2.6 μM and 172 μM, respectively. Both of these values are in close agreement to the apparent K_m's of 3 μM and 130 μM for PE and benzylamine, respectively, previously reported for MAO from human platelets, which have only the type B enzyme [5]. Furthermore, the relative activity towards these two B-specific substrates for hamster brain and human platelets are similar, i.e. the ratio of the V_{max}'s for benzylamine:PE is 2.2 for hamster brain and 1.4 for human platelets.

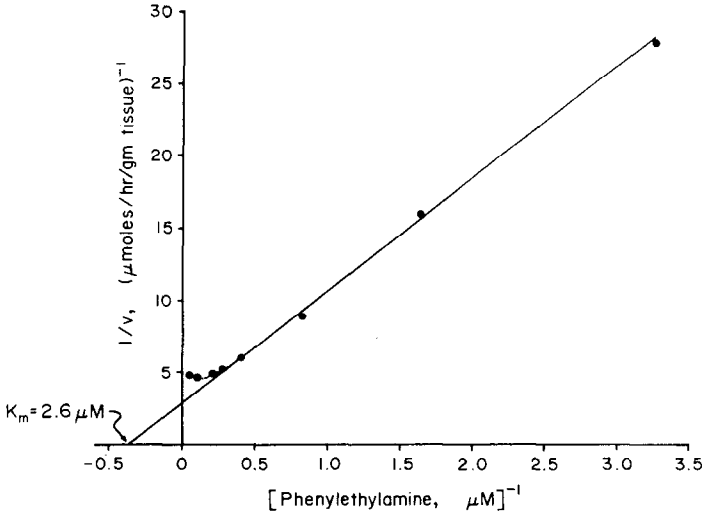

Fig. 3. Lineweaver–Burk plot for hamster whole brain homogenates using the substrate, PE (30 min incubation). Concentrations of substrate varied over the range of 0.304–19.5 μM.

Measurement of MAO activity in hamster platelets. No enzymatic activity was detected in isolated hamster platelets when either 5-HT or PE was used as substrate.

DISCUSSION

To our knowledge, this study represents the first report of the characteristics of MAO in the golden hamster. These data show that at least two forms of MAO are present in hamster brain and that they may be classified as types A and B forms of MAO according to the criteria originally used by Johnston [1]. That is, type A MAO is inhibited by low concentrations of clorgyline and uses 5-HT as a specific substrate; type B MAO, on the other hand, is inhibited by low concentrations of deprenyl but not clorgyline and shows selectivity towards the substrates PE and benzylamine. In this respect, hamster brain MAO is similar in its characteristics to MAO in rat and in several other mammalian species studied to date [2]. Some exceptions have been noted, however, for species which either appear to lack different forms of MAO or which have multiple enzyme forms but with different substrate specificities. It has previously been reported that distinct forms of MAO cannot be demonstrated in bovine mitochondrial preparations [7]. Oreland [8] has shown that pig liver contains two distinct forms of MAO but in this species 5-HT is oxidized by both types A and B MAO.

In agreement with previous work, our data suggest that the ratio of MAO-A/MAO-B activity can vary greatly among different organs even in the same species. Tipton *et al.* [3] have recently summarized the distribution of A and B forms of MAO in various organs of the rat. Although our data (Table 1) are expressed in terms of the ratios of enzymatic activities with the selective substrates 5-HT and PE, they are comparable to the data given by Tipton *et al.* [3], in which the proportion of the A and B enzymes is given by the per cent of activity at which the plateau occurs in the clorgyline inhibition curves using the non-specific substrate tyramine. With either method, the A/B ratios in various rat organs decrease in the following order: spleen > kidney > brain > lung > liver.

However, inhibition curves obtained by using selective inhibitors with a non-specific substrate are not sensitive to detecting two forms of MAO, if the activity of one of the two forms accounts for less than 5 per cent of the total activity [9]. This is illustrated by studies of MAO in rabbit tissues [4, 10]. The clorgyline and deprenyl inhibition curves obtained for rabbit MAO using tyramine as substrate do not appear biphasic. Consequently, only the B-form of MAO can be detected in this species by the use of selective inhibitors alone. However, as shown by the data in Table 1, rabbit tissues deaminate both A and B specific MAO substrates. In fact, the enzymatic activity towards 5-HT in each rabbit organ is nearly as great as, or even exceeds, the activity measured with the type B substrate, PE. Similarly, only the A-form of MAO was detected in rat heart homogenates when the selective inhibitor clorgyline was used [11]. It is, nevertheless, apparent from Table 1 that PE is deaminated to a small extent by rat heart homogenates.

In the present study, we have used the selective substrates in combination with selective inhibitors to examine the characteristics of hamster brain MAO (Figs 1 and 2). This approach appears to be superior to using either the specific substrates or specific inhibitors alone to detect two forms of MAO, particularly when one form of the enzyme is greatly predominant. Thus, we have clearly demonstrated the existence of both types A and B MAO in hamster brain even though the rate of PE deamination is only 3 per cent the rate of 5-HT deamination under our assay conditions. If the deamination of PE were due to a non-specific action of MAO-A rather than to the presence of a small amount of MAO-B, the inhibition curves obtained with 5-HT and PE would be coincident whether clorgyline (Fig. 1) or deprenyl (Fig. 2) was used as the selective inhibitor. This is clearly not the case, since the I_{50}'s towards 5-HT and PE differed by 220-fold and 1360-fold when clorgyline and deprenyl were used, respectively. In fact, presumably it is because the relative amount of MAO-B in hamster brain is so low (and thus less deprenyl is required to irreversibly inactivate it) that the selectivity of deprenyl appears so great, as evident in Fig. 2. Thus, the ability to discriminate between the two enzyme forms may actually become better when they are present in greatly unequal amounts if selective inhibitors are used in combination with selective substrates rather than a non-specific substrate.

The remarkably low activity of type B MAO in hamster brain suggests that further comparative studies between this and other species might profitably be used to gain a better understanding of the physiological role of the type B enzyme. Moreover, since the hamster has only 18 per cent of the hepatic phenylalanine hydroxylase activity as compared to the rat [12], the hamster may provide a useful model for phenylketonuria, particularly with regard to the role of amines derived from phenylalanine.

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